

*ABSTRACTS*

**4<sup>TH</sup> ALGAL VIRUS WORKSHOP**

**17-21 APRIL 2005**

**AMSTERDAM, THE NETHERLANDS**

**Presenting author in bold**

## ORAL

### GENOMIC CHARACTERISATION OF *EMILIANA HUXLEYI* VIRUS STRAIN 86

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*Emiliana huxleyi* is a unicellular alga found throughout the world's oceans. It is best known for its immense coastal and open ocean blooms at temperate latitudes that can cover 10,000 km<sup>2</sup> or more. The size and intensity of these blooms makes *E. huxleyi* important for nutrient and CO<sub>2</sub> cycling and biogenic sulphur production (in the form of dimethyl sulphide) in the marine environment. Consequently it is a key species for current studies on global biogeochemical cycles and climate modelling. We have isolated several strains of viruses that infect *E. huxleyi*. They are large double-stranded DNA viruses with genomes of approximately 410 kbp. We aimed to sequence the genome of one of these viruses (EhV86), characterise it and develop an oligo-microarray to determine temporal transcription profiles of the virus both *in vitro* and *in situ*. The 407kb genome of EhV86 has been sequenced in its entirety. The linear genome is 40.25% G+C and contains 467 putative genes. Only 20% of these genes contain any significant database homology. Of the known gene homologues, most are eukaryotic but there are also archeal, prokaryotic and virus homologues. Incredibly, there are only 14 genes in common between EhV86 and 2 other algal virus genomes from the same family of viruses. A first generation virus oligo-microarray was fabricated using 75-mer oligonucleotides corresponding to the sense strand of each virus ORF. A range of infection experiments were conducted to determine optimum conditions for extracting maximum quality & quantity of virus RNA. Our results revealed that the optimal time point for RNA extraction is 33h post-infection. Early results have shown that at least 50% of the virus genes are expressed 33h post-infection. This work was funded by NERC (Grant ref. NER/T/S/2002/00019).

## ORAL

### THE ISOLATION OF A FILAMENTOUS CYANOBACTERIUM AND ITS CO-OCCURRING RNA-CONTAINING VIRUS FROM A FRESHWATER LAKE.

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Cyanophages are a group of viruses that infect the globally important primary producers, the cyanobacteria. They are considered to play major roles in influencing host community diversity, terminating toxic cyanobacterial water blooms and influencing biogeochemical cycles. Numerous cyanophages have been isolated from both freshwater and marine systems, that exhibit typical bacteriophage morphologies possessing double-stranded DNA genomes. We have isolated a filamentous cyanobacterium of the family Oscillatoriaceae and also its co-occurring virus from a freshwater lake in the English Lake District, U.K. Work has been undertaken to characterise this virus with preliminary evidence suggesting this virus possesses a single-stranded RNA genome of 3500 nucleotides. The discovery of this virus suggests that cyanophages infectious to freshwater cyanobacteria are much more diverse than previously imagined.

## ORAL

CONTRIBUTION OF VIRAL INDUCED MORTALITY TO THE TERMINATION OF *PHAEOCYSTIS GLOBOSA* SPRING BLOOM.

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*Phaeocystis globosa*, a HAB-species, is known for its large spring blooms in temperate coastal waters of the North Sea. *Phaeocystis* is a genus with global distribution and significance for organic carbon and nutrients fluxes as well as studies on global warming. Although it has been shown that cell lysis accounted for up to 75% of the decline of these blooms, the contribution of viral infection has not been estimated before. Our study of a natural spring bloom of *P. globosa* showed that the concentration of viruses infecting *P. globosa* (PgV) increased towards the end of the bloom, concomitantly to enhanced phytoplankton cell lysis rates. Whereas grazing was the main mortality factor during the development of the bloom, viral mortality rates were highest during the wane of the bloom. Therefore we conclude that virally induced lysis is a significant mortality factor contributing to the decline of *P. globosa* blooms. Furthermore, virus isolation and characterization suggests a high diversity in PgVs, indicating viral regulation of *P. globosa* diversity on an intraspecies level.

## ORAL

A ROLE FOR CASPASES DURING VIRAL INFECTION OF THE COCCOLITHOPHORID, *EMILIANA HUXLEYI*

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Viral infection and programmed cell death represent two disparate modes of phytoplankton mortality. PCD is a refined, intrinsic cellular mechanism of active death, employing specialized cysteine proteases, caspases, which are fundamental in both its initiation and execution. The cellular mechanisms executing lytic viral infection in phytoplankton are not well understood, nor is it currently known whether viral infection shares mechanistic similarities with PCD. We examined how *Emiliana huxleyi* cells die when infected with the lytic virus, EhV1, specifically testing the involvement of caspases. EhV1 infection produced a dramatic reduction in the photosynthetic efficiency ( $F_v/F_m$ ) of *E. huxleyi* and a concomitant induction of caspase activity. *In vitro* caspase activity assays measured up to 200-fold elevation, increasing with progression of infection. Caspase activation was also confirmed through direct staining of *E. huxleyi* cells with a fluorescently labeled caspase inhibitor and subsequent flow cytometry analysis. Both assessments of caspase activity strongly correlated with mortality rate. Direct addition of a caspase inhibitor to a culture at the onset of infection inhibited both cellular caspase activity (60 to >90%) and limited viral infection. The inhibited culture had 3-fold higher cell concentration, higher  $F_v/F_m$ , and a 60-90% reduction in viral production. Gene expression levels of a caspase ortholog relative to actin, as determined by quantitative-RT-PCR, were elevated 24 h after initiation of infection, compared to uninfected controls. Our findings indicate that viral infection activates genetic and biochemical components of PCD machinery, perhaps playing an active role in its establishment and maintenance.

## KEYNOTE LECTURE

### METHODS IN VIRAL ECOLOGY

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Our knowledge about the properties of algal viruses in natural aquatic environments, their ecology and ecological significance is based on, and limited by, the methods we have developed and applied in our strive to describe and understand nature. A number of different methods and protocols for isolating new viruses, for detecting and quantifying viruses and their hosts, and for assessing their activity have been developed, tuned and tested. Some approaches and methods yield comparable results while others do not. In any case, the results and our interpretation may be biased by the method. The purpose of this presentation is to compare and discuss current methods in an attempt to tell facts from fiction.

## KEYNOTE LECTURE

### VIRAL CONTROL OF PHYTOPLANKTON

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Given the fact that photosynthetic organisms are at the base of every aquatic food web, it is to be expected that viral infection of algae impacts not only the host's population dynamics but also food web functioning and structure. Former years of research have shown that many different algal species are indeed sensitive to infection by viruses, that the virus-host interactions can be very specific, that algal viruses are diverse, and that many of algal viruses belong to the largest viruses studied so far. The field has steadily proceeded and the present time is an exciting one with new developments and progressing insight into the ecological role of algal viruses! The current presentation will show data of virally mediated mortality of phytoplankton (from laboratory culture work as well as field study sites), look into the role of viral control of phytoplankton populations, focus on factors affecting viral control, and last but not least discuss to what extent viral lysis of phytoplankton influences the organic matter flux and subsequently the pelagic food web.

## POSTER

### DETECTION OF PROGRAMMED CELL DEATH INDUCED BY VIRUSES IN MARINE PHYTOPLANKTON

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Traditional views of phytoplankton in nature consider that mortality is due only to grazing or sedimentation. It is now accepted that pathogens (e.g. viruses) are also important, but there are also reports of cell losses that cannot be attributed to specific factors. There have been major advances in understanding cell death in metazoans, specifically, the process of apoptosis, where the cell actively participates in its own death. Although the evolutionary meaning of such a process in unicellular organisms remains unclear, it is widespread in bacteria, yeast and protozoa, including at least two species of phytoplankton. Determining how phytoplankton die in nature is important because different fates have very different biogeochemical implications. We currently lack both an understanding of the relative importance of apoptosis and viral lysis in nature, and the techniques to measure and distinguish these processes. We have made measurements of general proteolytic activity in different virus-algal host model systems. Increased activities were noted in viral-infected cultures, but results differed between species. A more detailed study was undertaken using three flow cytometric assays of apoptosis which were adapted for marine phytoplankton: detection of membrane inversions (using annexin-V), DNA nicks (using fluorescent end-labelling) and caspases (using a fluorescently-labelled inhibitor). The methods were initially developed using the chlorophyte, *Dunaliella tertiolecta*, a species known to undergo apoptotic cell death when deprived from light. When applied to the prymnesiophyte, *Phaeocystis globosa*, undergoing viral infection, there was evidence of increases in caspases, but no consistent evidence of apoptosis using the other two markers. Protease activity and caspases may serve as an indicator of both stress- and virally-induced cell death, while additional cell markers could help to distinguish between these types of mortality.

## ORAL

### CONSTRUCTION MICROALGAE TRANSFORMATION VECTORS USING PROMOTERS FROM *CHLORELLA* VIRUSES AND COMPARISON OF THEIR ACTIVITIES IN TRANSFORMED *CHLORELLA* CELLS.

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*Chlorella* offers many advantages as an expression system of foreign proteins and recent expression of the flounder growth hormone in biologically active form suggest possible application of this system. In an attempt to improve the expression system, we replaced the CaMV 35S promoter with promoters from the tRNA gene clusters and early genes of chlorella viruses isolated in Korea. The tRNA gene cluster promoter regions are composed of repeated and conserved DNA motifs called Box A of 33 nucleotides and Boxes B or C of 84 nucleotides, which are arranged as AA in SS-1, CABA in KH-1, KH-2 and ABABA in SS-2 strain. Chlorella cells transformed with vectors containing ABABA-GFP fusion showed the strongest (110%) activity followed by AA-GFP fusion (108%) and CABA-GFP fusion (102%) compared to CaMV 35S-GFP fusion. Putative promoter regions of the DNA polymerase, DNA ligase and chitinase genes were amplified from 8 *Chlorella* viruses. Sequence analysis of these early gene promoters showed high sequence homology to the corresponding regions of PBCV-1 genome and the presence of many cis-acting elements for transcription factors. Chlorella cells transformed with the chitinase, the DNA polymerase and the DNA ligase gene promoter-GFP fusion construct showed 101.5%, 100.8%, 95.8% fluorescence intensity, respectively compared to CaMV 35S-GFP fusion. These results indicate that promoters from chlorella viruses are active in transformed chlorella, and chlorella viruses can be useful sources of promoters in addition to many genes of interest.

## POSTER

### COMPLETION OF CLONING AND NUCLEOTIDE SEQUENCE ANALYSIS OF THE SMALL SIZE GENOME OF FsV.

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FsV (*Feldmannia* sp. virus) is a member of phaeovirus genus in the *Phycodnaviridae* family, which infects filamentous marine brown alga, *Feldmannia*. FsV contains two virus groups distinguished by the size of their dsDNA genomes of 158kb and 178kb whose abundance in viral preparation is affected by culture temperature. A cosmid library has been constructed for the small size genome (Virology 220, 267-273), but there has been a gap, and only part of the genome has been determined. In this study, we have completed the cloning and nucleotide sequence analysis of the small size genome. Two PCR primers located at 500 nucleotides from each side of the gap produced a PCR product of 2.2kb, which cover the 1.2kb gap of the previous library. Sequence analysis of this PCR product showed the presence of two direct repeats that composed of 100 nucleotides and separated by 918 nucleotides. Between these direct repeats, two directly connected inverted repeats of 314 nucleotides are present. Complete nucleotide sequence of the genome including the gap has been determined. The viral genome is circular form and composed of 153,263 nucleotides with 48.2% A+T content. The genome contains at least 215 ORFs and some regions of repeat sequences that cover 6.7% of total genome. Among the 215 ORFs, 125(58.1%) ORFs have matched homologues in database, which include transposase, DNA polymerase, exonuclease, deaminase, sentrin, tRNA synthetase, protein kinase. FsV has 109(50.7%) and 84(39.1%) ORFs that matched to the ORFs of *Feldmannia irregularis* virus (FirrV) and *Ectocarpus siliculosus* virus (EsV), respectively. However, only 5(4.2%) ORFs showed similarities to the ORFs of *Chlorella* virus PBCV-1.

## ORAL

### IN SEARCH FOR GIANT VIRUSES

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The serendipitous discovery of Mimivirus, the largest known dsDNA virus (Science 2003 299: 2033), followed by the determination of its 1.2 Mb genomic sequence (Science 2004 306: 1344-50) revealed a new type of microbe the complexity of which is filling the gap previously thought to separate the largest viruses from parasitic cellular organisms. Detailed and robust phylogenetic analyses suggested that the common lineage leading to mimivirus and other extant Nucleocytoplasmic Large DNA viruses (NCLDVs) originated prior the emergence of the various eukaryotic kingdoms, and perhaps before that. Studying the genome of giant viruses is thus a new way of approaching the still mysterious process at the origin of the primordial eukaryotic cell. Following a presentation of the most original features found in Mimivirus genome, I will try to convince your expert audience that searching for more giant viruses is a worthwhile endeavour, a good strategy to understand their origin, and that algae and marine protists might be good candidates for hosting them.

## ORAL

### ESV-1 GENOME IS INTEGRATED INTO ITS HOST GENOME AS A SERIES OF SMALL DNA PIECES

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Phaeoviruses infect marine filamentous brown algae. They share an icosahedral structure with a lipid membrane, large dsDNA genomes, and a lysogenic life cycle. The DNA structure and the mechanism of integration of the *Ectocarpus siliculosus* Virus (EsV-1) are currently investigated. The DNA sequence of the EsV-1 genome was shown to be a linear molecule of 335,593 bp ended by large repeats, ITRA and ITRA'. In fact, several lines of evidence indicate that the EsV-1 genome has a chromosome-like structure including the presence of telomeres but that they are, nevertheless, able to form a circular molecule. It is likely that such a telomere resolution site is present in the region of the EsV-1 genome containing ITRA and ITRA'. An EsV-1 genomic library has been constructed in Stratagene's SURE 2 *E. coli* cells and screened. A DNA fragment containing this putative telomeric structure has been cloned and analysed confirming that the EsV-1 genome is also circular. Surprisingly, the circular molecule is 333,783 bp in length whereas the linear form is 335,593 bp in length. Another genomic library has been constructed using DNA of an EsV-1 infected *Ectocarpus* strain, NZVicZ14. This library has been screened with <sup>32</sup>P-labelled EsV-1 DNA and several clones that contain both viral and algal DNA have been isolated. Preliminary results indicate that the EsV-1 genome integrates into its host genome in one macro locus as a series of small pieces which are then reassembled when the virus becomes lytic in the reproductive structures of the alga.

## ORAL

### ANALYSES OF THREE CHLOROVIRUS VIRIONS

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The Chloroviruses [type member *Paramecium bursaria* chlorella virus-1 (PBCV-1)] are members of the Phycodnaviridae and are large, double stranded DNA viruses that infect chlorella-like green algae. The genome of PBCV-1 consists of 330,744 bp encoding for about 375 proteins and 11 tRNAs. Recently two additional virus genomes have been determined, namely the Chlorella NC64A infecting virus NY-2A and the Chlorella Pbi infecting virus MT325. Purified virions of these viruses were analyzed using both one dimensional SDS-PAGE and 2-dimensional IEF-SDS-PAGE, followed by in-gel trypsin digestion and separation of tryptic fragments with reverse phase HPLC, then sequence characterization with electrospray ionization-quadrupole-time-of-flight mass spectrometry. The mass spectrometry sequence data were compared to the NCBI total database for PBCV-1 and the newly derived sequence data bases for NY-2A and MT325. These analyses revealed about 120 unique virus-encoded polypeptides associated with the virions (approximately 1/3 of the coding capacity of these viruses), and a few non-virus-encoded polypeptides. Some of these polypeptides appear to be structural, whereas others appear to be enzymatic, mediating chromatin modification and signal transduction. The genes encoding these polypeptides are dispersed throughout the genome and are (where known) transcribed late in the infection cycle, which is consistent with virion morphogenesis. The long term goal of this study is to develop a comprehensive understanding of the Chlorovirus particles, including complete characterization of the polypeptides associated with the virion. These findings complement ongoing structural studies, as well as studies to understand the immediate-early events of host-virus interaction.

## ORAL

### IMMEDIATE-EARLY EVENTS IN PBCV-1 INFECTION OF *CHLORELLA* NC64A CELLS: A CONFOCAL LASER SCANNING MICROSCOPY ANALYSIS

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The immediate-early events of certain virus infections result in changes of cellular structure and function. For example, *Paramecium bursaria* chlorella virus-1 (PBCV-1) (type member of the Phycodnaviridae) infects chlorella-like green algae, and results in the rapid alteration of nuclear functions while initiating viral transcription. The attacking virion encapsidates the 330 kbp DNA viral genome containing closed inverted terminal repeats, as well as many virus-encoded proteins. Upon infection there is a rapid shift to viral transcription. However, the virus appears to depend on the cellular DNA-dependent RNA polymerase function for transcription, because it neither encodes for an RNA polymerase, nor is there evidence that it encapsidates a host RNA polymerase. Nevertheless, viral transcripts are apparent within 5-10 minutes of infection. It remains unknown where in the cell viral transcription is occurring. Using cell-impermeant fluorescent DNA-binding dyes that do not interfere with virus replication, PBCV-1 was labeled and used in both time-specific and real time infections. These infections were monitored with confocal laser scanning microscopy. Chlorella nuclear DNA was labeled with a cell-permeant DNA binding fluorescent dye, while the chloroplast was detected by autofluorescence of the intrinsic chlorophyll. Fluorescently tagged viral DNA enters the cell within 2 min of infection. The intracellular position of the incoming viral DNA was determined with XY, XZ and XYZ scanning, combined with sequential laser activation. Surprisingly, initial experiments indicate that the virus DNA resides within the cytoplasm of the infected cell during the immediate-early phase of infection, when early virus transcription occurs.

## POSTER

### PBCV-1 VIRION ASSOCIATED NUCLEASES

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PBCV-1 virus is a large, double stranded DNA virus (~330Kbp), that infects a unicellular, eukaryotic chlorella-like green alga. PBCV-1 genome encodes ~375 proteins + 11 tRNAs. Proteomic analysis revealed about 125 virion associated polypeptides, including type II restriction endonucleases CviAI and CviAII. Pulse field gel electrophoresis studies showed that PBCV-1 infection causes significant chromatin degradation within minutes after infection. Viral DNA is not degraded during infection. The presence of cycloheximide (translation inhibitor) has no effect on the ability of PBCV-1 to degrade the host chromatin. These results indicate that the virus does not require *de novo* protein synthesis for host DNA degradation, and that virion packaged proteins are associated with host chromatin degradation. Restriction endonucleases CviAI and CviAII are possible candidates to cause chlorella DNA degradation. PBCV-1 DNA is protected from CviAI and CviAII nucleases by site-specific modifications. However, exposure of purified PBCV-1 DNA to sonicated PBCV-1 preparations cause viral DNA degradation. This finding indicates that PBCV-1 packages some other nuclease(s) that can degrade PBCV-1 DNA. Possible candidates for the general nuclease include ORFs A227L and A177R, in as much that these putative nucleases are associated with virus particles. We hypothesize that DNases, including a general nuclease, are released into the nucleus during infection, but are sequestered from the viral DNA. The mechanism for this separation is unknown.

## ORAL

### PRODUCTION AND POTENTIAL ROLE OF DMS AND RELATED COMPOUNDS DURING THE VIRAL INFECTION OF ALGAE

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Dimethyl sulphide (DMS) constitutes the major source of biogenic sulphur to the atmosphere over remote ocean regions and can influence climate via aerosol formation and the subsequent production of cloud-condensation nuclei. The primary source of DMS is dimethylsulphoniopropionate (DMSP) a compatible solute found in certain marine phytoplankton. Equimolar amounts of DMS and acrylic acid are formed when DMSP is cleaved by phytoplankton DMSP lyase isozymes. It has been suggested that DMSP is also the base of an antioxidant cascade involving DMS, acrylic acid and their downstream products, including dimethylsulphoxide (DMSO). DMSO may also be biosynthesised by phytoplankton cells. The aim of this study was to follow the production and fate of these compounds during viral infection of axenic cultures of the phytoplankton *Emiliana huxleyi*. DMSO concentrations increased rapidly after viral addition. DMS and acrylic acid production occurred simultaneously during the later stages of the culture crash, but DMS concentrations were lower than those of acrylic acid suggesting that some of this pool may have been converted to another compound. Further culture studies revealed that during infection cells produce hydrogen peroxide and exhibit elevated levels of reactive oxygen species which supports the idea that some of the DMS produced during viral infection could act as an antioxidant. DMS production during viral infection of *E. huxleyi* was also observed during a mesocosm study in Raunefjorden, Norway in June 2003. Additional lab experiments indicate that DMS and acrylic acid reduce infective virus titres. The possible implications for defence against viral infection will be discussed.

## ORAL

### SEQUENCE ANALYSIS OF TWO LARGE CHLORELLA VIRUSES

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*Paramecium bursaria* chlorella virus (PBCV-1) is the prototype of a family of large, icosahedral, plaque-forming, double stranded DNA viruses that replicate in a unicellular, eukaryotic chlorella-like green algae, *Chlorella* NC64A. The PBCV-1 330-kb genome contains ~375 protein-encoding genes and 11 tRNA genes. Many of the gene products are unexpected for a virus, including enzymes involved in protein glycosylation and DNA restriction endonucleases. Another virus that infects *Chlorella* NC64A (NY-2A) and a virus that infects *Chlorella* Pbi (MT325) virus have been sequenced. The NY-2A genome consists of 368,683 bp making it the third largest viral genome sequenced to date. The genome contains ~400 protein-encoding genes. The MT325 genome is smaller than NY-2A and PBCV-1 with 314,335 bp containing ~330 protein-encoding genes. Since all three viruses are members of the family Phycodnaviridae, it's not surprising that they have many common protein-encoding genes. For example, these viruses encode for their own DNA polymerase, two PCNA's and one subunit of replication factor C. In addition, they encode for several enzymes involved with nucleotide metabolism. All three viruses encode for putative enzymes involved in transcription such as: three transcription factors, RNA guanylyltransferase, RNaseIII, and helicases; however, no RNA polymerase has been detected. As with PBCV-1, these two viruses encode unexpected proteins. For example, MT325 has the first functional aquaglycerolporin coded by a virus.

## ORAL

### DYNAMIC MODELLING OF CYANOPHAGE – HOST INTERACTION IN WHOLE WATER-COLUMN EXPERIMENTS

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Potential impacts of viruses on plankton communities in shallow, eutrophic lakes is of special interest since whole water-column experiments repeatedly showed collapse of the predominant filamentous cyanobacteria. The collapse could be associated with viral activity on a basis of electron microscopy of infected cyanobacterial cells and viral dynamics. Similar mass mortality did not occur in the original lake, but sudden clearing with disappearance of cyanobacteria has been observed elsewhere. Experimental results indicate that viral outbreak can be triggered by unusually high external nutrient supply. High-nutrient status of the host cells might accommodate a large burst size, i.e., high number of viruses assembled per cell. Burst size effect on single-host single-virus dynamics was modelled for nutrient-replete growth of the cyanobacteria and fixed viral decay rate in the water column. According to the model results, burst sizes greater than 150 would result to host extinction, whereas lower numbers would allow coexistence, and even stable population densities of host and virus for a burst size around 50. The ecological implication is that burst-size increase accompanying a transition from phosphorus to light-limited cyanobacterial growth might destabilize the cyanophage – host interaction. However, observations on cyanobacterial collapse in shallow lakes are scanty and lack details on viral infection as the mortality cause.

## POSTER

### FLOW CYTOMETRIC ANALYSIS OF BACTERIA AND VIRUSES IN LAKE SEDIMENTS

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Flow cytometry (FCM) was successfully used to analyse freshwater bacteria and viruses in lake sediments after sample treatment and optimization of dilution/fixation/staining procedures. Biological particles from Lakes Geneva and Bourget were first separated from the sediments by using both pyrophosphate (0.01 M final concentration) and TWEEN 80 (10% final concentration) and sonification during 10 min in a bath filled with both water and ice. The best results (highest virus and bacterial yields from the bulk of the sediments) were obtained with formaldehyde fixation within a few hours (2% final concentration, vs. non fixation or use of glutaraldehyde), SYBR-Green II staining (x 1/20,000 stock solution concentration, vs. use of SYBR-Gold, SYBR-Green 1 and SYTO 9 dyes at different concentrations), and no use of DNase 1. There was an important loss of particles after only a few days of preservation at 4°C or -22°C. For FCM analysis, samples were diluted in Tris-EDTA buffer (pH 8) and heated for 10 min at 80°C. Counts of bacteria and viruses were correlated with those obtained with epifluorescence microscopy but EFM gave always lower concentrations than FCM. The analysis of the distribution of the viruses in the water column and in the sediments of Lakes Bourget and Geneva revealed an important gradient with higher quantities in the sediment in the top layer than in the overlying waters of deeper in the sediment. It is likely that such a use of FCM could be applied for the detection of algal viruses in

**ORAL**

QUANTIFYING VIRAL VERSUS GRAZING MORTALITY USING THE DILUTION APPROACH: EFFECTS OF NUTRIENT ADDITIONS DURING A PHOSPHATE AND IRON ADDITION EXPERIMENT IN THE OLIGOTROPHIC, SUBTROPICAL, N. ATLANTIC.

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Lysis by viruses and grazing by protozoa represent two fundamentally different pathways by which carbon and nutrients may cycle within microbial food webs. Although we are beginning to understand the possible ecological role of marine viruses, it remains difficult to quantify the viral component of phytoplankton mortality, due to the lack of suitable techniques. In this presentation we will discuss the use of the dilution incubation approach as a method for determining the relative importance of viral lysis versus grazing as sources of phytoplankton mortality. We will also present data showing the interactions between viruses and cyanobacteria during a recent open ocean iron/phosphate enrichment study (FeEP). Results show how the microbial component responded to the nutrient addition and how changes in virus abundance appeared to be coupled to changes in cyanobacteria populations.

**ORAL**

SIMULTANEOUS PRODUCTION OF TWO DISTINCT VIRAL PARTICLES IN *HETEROSIGMA AKASHIWO*.

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The isolation and characterization of algal viruses from aquatic ecosystems has uncovered a rich assortment of novel viruses, including dsDNA, ssRNA, and dsRNA viruses. We have isolated a novel virus system that adds further to this diversity. In this system two distinct viral particles replicate in the cytoplasm of the host alga, *Heterosigma akashiwo*, at the same time and are released from the cell by lysis approximately 72 hours post-infection. Both particles contain DNA but differ in diameter, abundance, and genome size. The smaller, more abundant particle is approximately 50 nm in diameter and contains 20 kb of DNA. The larger particle is icosahedral, 160 nm in diameter, and contains approximately 180 kb of DNA. Methods for separating and purifying the two viruses are being developed. Preliminary results suggest that OptiPrep gradients provide optimal separation while retaining infectivity, and thus should allow experimental determination of the function of each particle in transmitting infection.

## ORAL

### A WHITER SHADE OF PALE: CORALS SING THE BLUES

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Over the past few years a sequence of major bleaching events has threatened the world's coral reefs. Coral bleaching manifests itself as a loss of zooxanthellae and/or chlorophyll from cnidarian hosts. The underlying cause of bleaching and the mechanisms involved are largely unknown. Here we investigate the hypothesis for bleaching that zooxanthellae harbour a latent virus. If zooxanthellae harbour latent viruses, environmental triggers, such as increased temperatures and UV light, will induce a lytic cycle which may lead to the deterioration and expulsion of the symbionts by their host and hence cause bleaching of the host animal. Evidence from transmission electron microscopy (TEM) and flow cytometry (FC) suggests that viruses are induced from the zooxanthellae. It is therefore possible that environmentally-regulated viral induction mechanisms contribute to bleaching events in coral reef environments. Ultimately, we plan to lay the foundations for the development of molecular probes to detect viruses in field samples through the isolation, characterisation and genomic sequencing of viruses present in the symbiotic zooxanthellae of corals.

## ORAL

### MOLECULAR DYNAMICS OF EhVS IN BLOOMS OF THE MARINE COCCOLITHOPHORID *EMILIANA HUXLEYI*

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Viruses play an important role in the life of plankton populations. Phytoplankton are at the base of both the food web and the microbial loop in the oceanic ecosystem. Consequently, the viruses that infect them are likely to significantly affect the structure, function and biogeochemical cycling of marine food webs. *Emiliana huxleyi*, a marine microalga that forms dense blooms in coastal and mid-ocean waters, is an important species with respect to past and present marine primary productivity, sediment formation and climate. Previous studies have shown the effectiveness of the calcium-binding protein (GPA) and the major capsid protein (MCP) genes to reveal significant sequence variation between *E. huxleyi* and their virus (EhV) strains. New denaturing gradient gel electrophoresis (DGGE) and sequencing primers, now available, have allowed us to check the dynamics by studying the composition of host and virus genotypes during the blooms. In the current study we have monitored the progression of three different blooms of *E. huxleyi*; a natural bloom in the North Sea in June 1999 and two induced blooms in seawater enclosures off western Norway in June 2000 and 2003. In addition to the genotypic analysis, flow cytometry (FCM), was used count the *E. huxleyi* and virus communities. Host and viral communities were more diverse and dynamic in the North Sea than in the induced experiments, and they showed temporal and depth variations. During the mesocosms we observed different bloom dynamics but yet the same community of host and viruses. Only 2 virus genotypes dominated during the *E. huxleyi* crash in the mesocosms. This suggests that only those were responsible for the termination of the bloom.

## ORAL

### HOW SIMILAR IS THE REPLICATION PROCESS OF PLANT AND ALGAL VIRUSES HAVING A POSITIVE-STRAND SS-RNA GENOME?

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The majority of land plant viruses have a positive-strand ssRNA genome, and share considerable similarities in RNA replication in spite of the varieties of genomic organization, virion morphology, and host range. In contrast to algal ssRNA viruses (HaRNAV, HcRNAV), plant ssRNA viruses require damage to the host cell membrane to gain entry into the host cell (e.g. bite by an insect). In plant ssRNA viruses, following uncoating, the positive-strand genomic RNA (messenger) is translated to yield the viral replication proteins. Because positive-strand ssRNA viruses lack the machinery for mRNA translation, viral protein synthesis is dependent on the host for translation. The viral replication proteins and host cell components assemble the RNA replication complex using the host's organellar membranes. (e.g. the membranes of the mitochondria and/or the endoplasmic reticulum). Adapting the viral RNA and replication proteins to the host components are likely the core determinants for the host range of the viruses. The mechanism of algal virus RNA replication is not known. In a preliminary experiment, HcRNAV genomic RNA was injected into the plant cells of *Vigna unguiculata* and *Nicotiana tabacum*; however, we found no replication of the HcRNAV genomic RNA. This suggests a lack of replication elements in these plants necessary for HcRNAV genome replication. In this paper, we introduce the general replication mechanism of positive-strand RNA viruses infecting land plants, and assess the possible application of land plant virus replication as a model to investigate replication in algal ssRNA viruses.

## KEYNOTE LECTURE

### A MOLECULAR ECOLOGY STUDY OF DINOFLAGELLATE AND VIRUS RELATIONSHIPS

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HcRNAV infects the bloom-forming dinoflagellate *Heterocapsa circularisquama*. Field data concerning the host alga and its virus populations indicate an intimate interrelationship occurs. HcRNAV strains show two types, UA-type and CY-type, based on complementary intra-species host specificities. The genome structure of the two types is very similar with a ~97% nucleotide sequence identity having two ORFs: ORF-1 codes for a polyprotein and ORF-2 codes for a single major 38 kDa capsid protein. Comparisons of the two types show four regions in the capsid protein amino acid sequences that have notable sequence differences. The tertiary structure of these capsid proteins predicted by computer modeling suggests the variable regions are on the outside of the particles. Hence, the intraspecies host specificity is most likely determined by surface nano-structures on the capsid protein that may affect the ability of the viruses to bind to a suitable host. A nested RT-PCR was designed to amplify the corresponding variable regions; and applied to purified RNAs from other HcRNAV strains and natural marine sediments. The results of this analysis show the types of these variable regions completely coincided with the intraspecies host specificities; and the sediment clonal sequences also showed considerable similarities to either type sequence. Using relatedness comparison, we concluded that subtle elegant molecular changes in the virus coat account for the host-virus relationships in the natural marine microalgal ecology. This study was supported by the Industrial Technology Research Grant Program in 2004 of the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

## ORAL

### PREVIOUSLY UNKNOWN TYPE OF VIRUS INFECTS A BLOOM-FORMING MARINE DIATOM *CHAETOCEROS SALSUGINEUM*

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Diatoms are a major phytoplankton group that plays important roles to maintain oxygen levels in the atmosphere and sustain the primary nutritional production of the aquatic environment. Among them, the genus *Chaetoceros* is one of the most abundant and widespread. Temperature, climate, salinity, nutrients, and predators were regarded as important factors controlling the dynamics of diatoms; here, we show that a viral infection can also be a mortality source for *Chaetoceros*. We isolated a DNA virus specifically infecting the bloom-forming diatom *C. salsugineum* and designated it “*C. salsugineum* nuclear inclusion virus (CsNIV)”. It is a 38 nm icosahedral virus that replicates within the host’s nucleus. CsNIV has a genome structure unlike that of other viruses that have been described; i.e., it consists of a single molecule of covalently closed circular single-stranded DNA (ssDNA, 6,005 nt) as well as a segment of linear ssDNA (997 nt). The linear segment is complementary to a portion of the closed circle creating a partially double-stranded genome. Sequence analysis reveals a low but significant similarity to the replicase of circoviruses that have a covalently closed circular ssDNA genome. However, viral replication mechanism including its codon usage is still unknown. This new host-virus system will be useful to investigate the relationships between diatoms and other viruses in aquatic systems. This study was partially supported by Grants-in-Aid for Scientific Research (A) (2) (No. 16208019) from the Ministry of Education, Science and Culture in Japan and the Industrial Technology Research Grant Program in 2004 from NEDO of Japan.

## POSTER

### CHARACTERIZATION OF CELL WALL ATTACHMENT PROTEINS OF CHLOROVIRUSES

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Chloroviruses are large icosahedral, dsDNA-containing viruses that infect certain strains of the unicellular green alga *Chlorella*. Using the plaque assay, these viruses are easily detected in natural freshwater from all over the world. Despite of their ubiquity, little is known about their natural hosts, infection cycle and molecular mechanisms of dissemination. We have been interested in identifying and characterizing chloroviral proteins that interact with the host cell wall, and have found that a protein Vp130 likely has such a function. Vp130 consists of a highly conserved N-terminal domain, internal repeats of 70-73 aa. motifs and a C-terminal domain occupied by 23-26 tandem repeats of a PAPK motif. However, CVG1, one of Pbi viruses, has a little different Vp130 where the shorter PAPK repeats are not the C-terminus but internal. Since NC64A viruses do not infect strain Pbi and strain NC64A does not serve as a host for Pbi viruses, this structural difference may be closely related to the discrimination mechanism. Recombinant Vp130 proteins formed in *E.coli* bound to the host cells, and competed between CVK2 and CVG1. Although recombinant proteins didn't show host-specific adsorption, these results suggest that Vp130, especially its repetitive region is essential for the adsorption of chloroviruses to their host cells.

## POSTER

### GIANT VIRUSES OF MARINE ECTOCARPOIDS CONTINUED...

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With the retirement of two eminent algal virologists, Prof. Dieter Müller & Prof. Russel Meints, the characterisation of the viruses that infect the different ectocarpoids has all but ceased. The ectocarpoids all fall within the order Ectocarpales, which currently includes 5 families namely, Ectocarpaceae, Scytosiphonaceae, Chordariaceae, Adenocystaceae and Acinetosporaceae. Viruses are known to infect the members within three families of the Ectocarpales - Ectocarpaceae, Chordariaceae and Acinetosporaceae. *Ectocarpus siliculosus* virus (EsV-1), *Feldmannia* sp. virus (FsV) and *Feldmannia irregularis* virus (FirrV-1) are the only members that have been studied in detail. Moreover, the sequencing of EsV-1 and more recently its host, *Ectocarpus siliculosus*, has and will continue to dominate the landscape of ectocarpoid virology. Currently, at least 5 other species of ectocarpoids are known to be infected by similar viruses. Most of these viruses have only undergone a basic form of characterisation and the relationship between all these ectocarpoid viruses has yet to be determined. Here we present some preliminary genotypic data elucidating this relationship.

## ORAL

### *HETEROSIGMA AKASHIWO* VIRUS STRAINS WITH DISTINCT INTRASPECIES HOST SPECIFICITIES HARBOR IDENTICAL INTEIN ELEMENTS

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*Heterosigma akashiwo* virus (HaV) is a large dsDNA virus infecting the bloom-forming raphidophyte *Heterosigma akashiwo*. Molecular phylogenetic sequence analysis of HaV DNA polymerase showed that it forms a sister group with several phycodnaviruses. All ten examined HaV strains, with distinct intraspecies host specificities, include in their DNA polymerase genes an intein (protein intron). The 232 amino acids inteins differed from each other by no more than a single nucleotide change. All inteins were present in the same conserved position, coding for an active-site motif (YGD/TDS), which also includes inteins in Mimivirus (a very large dsDNA virus of amoebae) and several archaeal DNA polymerases. The HaV intein is closely related to the Mimivirus intein and both are apparently monophyletic to the archaeal inteins. The homing endonuclease (EN) domain of the HaV intein alleles is mostly deleted; i.e., it is likely a remnant of a typical intein LAGLI-DADG homing EN domain that underwent a deletion of its central part. These observations suggest horizontal transfers of inteins between viruses of different families and between archaea and viruses, and that viruses might be reservoirs and intermediates in intein horizontal transmissions. The mechanism keeping their sequences basically identical in HaV strains specific for different hosts is yet unknown. One possibility is that rapid and local changes in the HaV genome change its host specificity. This is the first report of inteins found in viruses infecting eukaryotic algae. This study was partially supported by the Industrial Technology Research Grant Program in 2004 from NEDO of Japan.

## POSTER

### CHARACTERIZATION OF A SS-RNA VIRUS INFECTING THE BLOOM-FORMING DIATOM *RHIZOSOLENIA SETIGERA*

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*Rhizosolenia setigera* Brightwell is a centric diatom which widely distributes over the world, and known as one of the harmful-bloom forming diatoms that cause the discoloration of a seaweed “laver (*Porphyra yezoensis*)”. In this paper, we discuss the characteristics of a novel virus (RsRNAV) infecting *R. setigera*. The virion is icosahedral, lacking a tail, ca. 32±1 nm in diameter, and has three major proteins (ca. 41.5, 41.0 and 29.5 kD). The nucleic acid extracted from RsRNAV particles is single-stranded RNA (ssRNA) 11.2 kb in length; although smaller RNA molecules with sizes of 0.6, 1.2, and 1.5 kb were occasionally observed. Sequencing of the genomic RNA is now being performed (the central region has not yet been sequenced). NCBI Blast search for the 5' fragmental nucleotide end sequence revealed considerable similarities with a polyprotein of *Heterosigma akashiwo* RNA virus (HaRNAV), putative RNA-dependent RNA polymerases of unidentified picorna-like (marine) viruses, an unidentified chinese clam virus, Broad bean wilt virus, etc., and non-structural polyprotein of Taura syndrome virus. It is also notable that the 3' nucleotide end sequence showed a low similarity (E-value = 2E-3) to the HaRNAV polyprotein. These results suggest these marine and land viruses had originated from an identical ancestor virus group and expanded the host range through the process of evolution. This study was partially supported by Grants-in-Aid for Scientific Research (A) (2) (No. 16208019) from the Ministry of Education, Science and Culture of Japan and the Industrial Technology Research Grant Program in 2004 from NEDO of Japan.

## ORAL

### OPTICAL CHANGES ASSOCIATED WITH CYANOBACTERIAL BLOOM TERMINATION BY VIRAL LYSIS

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Optical changes that accompanied mass viral lysis of a community of filamentous cyanobacteria from the shallow, eutrophic Lake Loosdrecht (The Netherlands), were studied in a semi-natural system under controlled conditions. Knowledge of the optical properties of mass lysis may help to identify occurrences of virally induced mass mortality of cyanobacterial populations by means of optical remote sensing. Under experimental conditions, viral collapse of the dominant filamentous cyanobacterial population led to drastic optical changes. A marked increase in water transparency was related to decrease of the scattering coefficient by ≈ 80%, and decrease of the absorption coefficient by 20-80%. Mass lytic events and related optical changes were completed within one week after the first signs of viral lysis. Spectral characteristics of these changes were similar between experiments that were started in different seasons. Release of dissolved materials and submicron-sized particles exhibiting chlorophyll a absorption were observed upon lysis. A drastic decrease of the phycocyanin to chlorophyll a ratio and a simultaneous increase of the phaeopigment to chlorophyll a ratio were also observed. These optical changes indicate that mass mortality of this magnitude can be detected in natural systems.

## KEYNOTE LECTURE

PHYCODNAVIRUSES, CYANOPHAGES, AND MARNAVIRUSES: DOES VIRAL PHYLOGENY PROVIDE INSIGHTS INTO HOST POPULATIONS?

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As more sequence data become available from cultured viruses and from uncultured natural virus communities, a pattern frequently emerges of groups of relatively closely related sequences falling into well defined clusters. In the case of the Phycodnaviridae and Marnaviridae a good case can be made that these clusters are associated with viruses that infect a specific taxon of algae. This strongly suggests that the phytoplankton and the viruses that infect them are highly coevolved. The case for putative cyanophages is less convincing. Again, there are groups of closely related sequences that occur at the ends of branches; however, these sequences can be derived from samples collected from very different environments. This suggests that the hosts are not closely related and that these genes must be circulating relatively freely and widely in the environment. Moreover, it seems evident that some of these genes are shared amongst viruses that infect prokaryotes and eukaryotes; hence, lateral gene transfer amongst these viruses through host intermediaries appears rampant. Consequently, the co-evolutionary relationships between cyanophages and cyanobacteria might be quite different that for viruses infecting eukaryotic phytoplankton.

## POSTER

SYNECOCOCCUS GROWTH IN THE OCEAN IS DEPENDENT ON VIRAL LYSIS OF HETEROTROPHIC BACTERIA

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We investigated the effect of viruses on the growth of *Synechococcus* in the Gulf of Mexico and the Mediterranean Sea. Whole seawater was diluted with 0.2- $\mu$ m filtered (virus-rich) and 30 000 to 100 000 MW cutoff filtered fractions (virus-free) and the growth of *Synechococcus* was monitored in this fractions as well as in whole seawater. The frequency of dividing cells, growth rate and abundance were higher in whole seawater and in dilutions with viruses than in dilutions with virus-free water. In an experiment where viruses were heat-activated, FDC also increased in the presence of active viruses indicating that it was not organic matter in the virus-size fraction that stimulated growth of *Synechococcus*. Cyanophages were only a minor component of *Synechococcus* mortality (0 – 0.8%), whereas virus-induced mortality of bacterioplankton removed a significant portion of bacterial production. Our data suggest that viral lysis of heterotrophic bacteria regenerates nutrients, which can be taken up by *Synechococcus* and sustain its growth rates.

## ORAL

### GENOME STRUCTURE OF A MARINE FUNGOID PROTIST-INFECTING RNA VIRUS

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SssRNAV (previously reported as “ThV”) is a virus infecting *Schizochytrium* sp. strain N1-27 that belongs to the Labyrinthulomycetes (marine fungoid protists). The virus genome is a single-stranded, linear, positive-sense RNA, 9,018 nt in length (excluding polyA tail), which contains two large ORFs. The ORFs are separated by an intergenic region of 92 nt. The 5' ORF (ORF1) is preceded by an untranslated leader sequence of 554 nt, while the 3' large ORF (ORF2) and an additional smaller ORF (ORF3), which overlaps ORF2 by 429 nucleotides, are followed by an untranslated region of 70 nt (excluding polyA tail). The deduced amino acid sequences of ORF1 and ORF2 products, respectively, showed similarity to the non-structural proteins and structural proteins of dicistroviruses, which infect insects or shrimps. The phylogenetic analysis from the deduced amino acid sequences of RNA-dependent RNA polymerase (RdRp) and RNA helicase-RdRp complex suggest that SssRNAV is closely related to the family *Dicistroviridae* and also to HaRNAV (*Heterosigma akashiwo* RNA virus). Northern blot analyses suggested that SssRNAV synthesizes subgenomic RNAs to translate ORF2 and ORF3, suggesting that the translation mechanism for downstream ORFs is distinct from those of dicistroviruses. Integrating these results, we conclude that SssRNAV is not a member of currently defined virus families, and belongs to a new unrecognized virus group. Assuming that viruses hosting protists, microalgae, and crustaceans originated from an identical ancestor virus group and that they have expanded the host ranges over time and space, this kind of study leads to fascinating considerations on virus evolution process.

## ORAL

### GENOMIC ANALYSIS OF A NOVEL PHAGE INFECTING THE TOXIC CYANOBACTERIUM *MICROCYSTIS AERUGINOSA*

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This paper describes the preliminary genome characterization of Cyanophage Ma-LMM01 infecting the toxic cyanobacterium *Microcystis aeruginosa*. Phage genome was extracted from virions purified in a step gradient of cesium chloride. The genome is a linear dsDNA, the length of which was estimated at ~140 kbp by PFGE. Ma-LMM01 DNA was sensitive to all 14 restriction enzymes tested, suggesting a low level of methylation. Shotgun sequencing is now under way; so far, >100 ORFs (>300 nt in length) have been identified. Several ORFs showed considerable similarities to phage-related proteins: endolysin of *Pseudomonas* phage D3 (E-value: 4E-9), phage tail sheath protein FI of *Xylella fastidiosa* Dixon (7E-8), prophage antirepressor of *Clostridium thermocellum* (3E-19), etc. Ma-LMM01 genome also codes for a number of genes showing high similarity to various cyanobacterial proteins, ex. allophycocyanin-B, CTP synthase, ribonucleotide reductase, lipoprotein nlpD of *Synechocystis* sp., hydrogenase expression/formation protein, membrane-bound lytic transglycosylase A, transposase, and serine/threonine kinase of *Nostoc* sp. (*Anabaena* sp.). etc. Especially, an ORF showing strikingly high similarity (5E-89) with a putative site-specific integrase of *Synechocystis* sp. WH 8501 is of great interest. Further analysis of this gene would be meaningful to assess the lysogenic tactics of this phage. Our observation supports the idea of horizontal gene transfer by phages within cyanobacterial communities in natural waters. Besides, we expect further studies on cyanophage genomes would lead a rational explanation for the enigma why distinct cyanobacterial genera produce similar toxic compounds. This study was financially supported by NEDO of Japan.

## ORAL

### K<sup>+</sup>-CHANNELS OF CHLORELLA VIRUSES ARE RESPONSIBLE FOR MEMBRANE DEPOLARIZATION OF HOST CELLS IN EARLY STAGE OF INFECTION

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Previous studies have established that chlorella viruses encode K<sup>+</sup> channels with different structural and functional properties. In the current study we exploit the different sensitivity of these channels to Cs<sup>+</sup> to determine if the membrane depolarization observed immediately after virus infection is caused by the activity of these channels. Infection of *Chlorella* NC64A with four viruses caused rapid membrane depolarization of similar amplitudes but with different kinetics. Depolarization was fastest after infection with virus SC-1A ( $t_{1/2}$  about 8 min) and slowest with virus NY-2A ( $t_{1/2}$  about 12 min). Cs<sup>+</sup> inhibited membrane depolarization only in viruses that encode a Cs<sup>+</sup> sensitive K<sup>+</sup> channel. Collectively, the results support the hypothesis that membrane depolarization is an early and essential process in chlorella virus/host interactions and that it is correlated with the activity of the viral channel. This correlation also implies that the virion contains the channel prior to infection, presumably in its internal membrane. Fusion of the virus membrane with the host plasma membrane results in an increase in K<sup>+</sup> conductance and membrane depolarization. This depolarization can also explain how chlorella cells infected with one virus resist infection by a second virus.

## ORAL

### SEASONAL VIRAL, BACTERIAL AND CYANOBACTERIAL POPULATION DYNAMICS IN A SHALLOW, EUTROPHIC LAKE.

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We describe the seasonal dynamics of virioplankton in a filamentous-cyanobacteria-dominated lake. The virioplankton community in this shallow, eutrophic lake (Lake Loosdrecht, The Netherlands) is especially interesting since earlier research repeatedly showed a dramatic collapse of the cyanobacterial community associated with viral activity during lake water enclosure experiments. Furthermore the presence of virally infected cyanobacteria in Lake Loosdrecht has been observed and it is known that grazing only accounts for part of the cyanobacterial mortality. These observations suggest that the viral community could play a significant role in shallow, eutrophic lakes. During March till November 2003, samples were taken every two weeks to describe the virioplankton community, its possible hosts and physical parameters. Cyanobacterial numbers were observed to be high year round, peaking strongly in June. The strongest increase in viral growth occurred during this cyanobacterial peak. Diversity of the virioplankton, bacterial and cyanobacterial populations was observed using PFGE and DGGE. Temporal changes in the diversity and composition of the virioplankton and the bacterial and cyanobacterial populations were apparent. Viral populations with genome sizes varying from 20 to 150 kb were detected. The majority of the viral population had genome sizes clustering around 45 and 60 kb year round. Virioplankton diversity was observed to be highest in winter. The environmental variables controlling viral abundance and the importance of the virioplankton community in Lake Loosdrecht will be discussed. We will also discuss the possible role of flagellates in the decay of the viral community in this lake.

## POSTER

### WHY HCRNAV ALLOWS PARTIAL SURVIVAL OF ITS HOST CELLS?

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When HcRNAV is inoculated into clonal cultures of *Heterocapsa circularisquama* (Dinophyceae), part of the host cells survive and re-grow. The objective of the present study is to determine the mechanism of why partial survival of *H. circularisquama* occurs. We isolated 100 cells from a clonal host culture (not exposed to HcRNAV after establishment from natural water) and tested virus sensitivity of each host clone. One virus-sensitive host clone was maintained using a virus-free medium (NV). Three hundred cells (in total) were intermittently isolated out of it and tested for their virus sensitivity. We also isolated 100 cells out of those surviving in a virus-inoculated culture, and a virus sensitivity test was performed; then, one surviving clone was independently maintained with a virus-containing medium (SV) or with a virus-free medium (SN). In the same manner, a virus sensitivity test was performed. We found the virus sensitivities of NV, SV, and SN cells were high (100%), low (<7%), and changeable (29-97%), respectively. These results indicate that (1) in a clonal culture, a small portion of algal cells are likely resistant to viral infection; (2) the resistance can be lost when there is no virus-pressure (no virus present); and (3) the resistance can be retained under virus-pressure (virus present). It is unlikely that a mutation causes the survival of the host where possibly the resistance that the host demonstrates naturally has this ability. This study was partially supported by the Industrial Technology Research Grant Program in 2004 from NEDO of Japan.

## POSTER

### FLOW CYTOMETRY TO COUNT MICROALGAL VIRUSES HAVING A DS-DNA OR SS-RNA GENOME

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Flow cytometric (FCM) counting of algal viruses in culture and in natural samples have recently been improved. Here, we show further-optimisation are required to detect and count particles of each individual virus species by FCM. The optimum conditions to count two phycodnaviruses, HaV (*Heterosigma akashiwo* virus, 0.2µm, 294 kbp) and HcV (*Heterocapsa circularisquama* virus, 0.2µm, 356 kbp), using FCM were determined. Although HcV stained well and was detected using SYBR-Green I at a  $1 \times 10^{-4}$  dilution of the commercial stock, the green fluorescence (GFL) of similarly stained HaV particles was less. To increase sensitivity for HaV, addition of Triton-X (0.1%) and heat-treatment (80°C, 10 min) were effective. Counting each virus using the optimum staining method compared highly to using epifluorescence microscopic counts at densities of  $>10^3$  particles ml<sup>-1</sup> ( $r = >0.999$ ). Conversely, fixation and freezing decreased the GFL and counts of the viruses. Besides, we similarly stained the small RNA viruses infecting microalgae: HcRNAV (30nm, 4.4 kb) and RsRNAV (30nm, 11.2 kb). RsRNAV was detectable by SYBR-Gold staining. However, the GFL was weak, and the counts were lower than the MPN values; we do not have a reasonable explanation for these results. Although we have improved FCM counting for two large microalgal dsDNA viruses, improvement of staining technique will be required to count algal viruses having ssRNA genome. This study was partially supported by the Ministry of Agriculture, Forestry and Fisheries of Japan and the Industrial Technology Research Grant Program in 2004 from NEDO of Japan.

## POSTER

### VIRUSES AND ZOOPLANKTON, MORTALITY FACTORS FOR FRESHWATER CYANOBACTERIA

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Cyanobacteria are photoautotrophic prokaryotes that can grow to dense populations in eutrophicated freshwater lakes, the so-called blooms. These blooms can cause large economic damage in shallow recreational lakes due to deterioration of the water quality, deoxygenation, their toxicity, foul odours and the resulting decrease in aesthetic value. Besides zooplankton grazing as possible control agent for these cyanobacteria, it is suggested that also viral lysis can play an important role in cyanobacterial mortality. Viruses have been reported to have the ability to terminate cyanobacterial blooms. Not only are viruses interesting in respect to collapsing cyanobacterial booms, the impact viral lysis can have on aquatic foodwebs differs in respect to the impact zooplankton grazing can have. The general aim of this research was to quantify virus- and grazing-induced mortality rates of the cyanobacterial community in a Dutch eutrophicated shallow peat lake. To do so, the dilution method was used, which was originally meant for determining zooplankton grazing activity (Landry & Hassett, 1982) and adapted by Evans *et al* (2003) to make it applicable for viral lysis quantification. The method was successfully applied on eutrophic lake water and viral induced mortality of cyanobacteria and heterotrophic bacteria during the winter season could be determined. No mortality however was observed due to grazing, which was possibly caused by low zooplankton numbers in winter. Viruses removed up to 54% of cyanobacterial production, while for heterotrophic bacteria this was even 91%. These results show that viruses remove an important proportion of cyanobacterial production.

## KEYNOTE LECTURE

### INS AND OUTS OF A QUARTER CENTURY OF ALGAL VIROLOGY

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The talk will briefly cover some of the significant events (as judged by this speaker) in the development of the algal virus field. I will then update some newer findings on the chlorella viruses, including the fact that the algal viruses probably have a long evolutionary history going back to the time eukaryotes and prokaryotes separated (maybe 3 billion years ago). Using the chlorella virus encoded potassium ion channel protein as an example, I will illustrate how this long evolutionary history can be exploited to study structure/function relationships of the protein. Finally, I will speculate on why I think these large dsDNA viruses may become even more important in the field of Virology in the next 10 years.

## KEYNOTE LECTURE

### BERRY-STONE VIRUSES: 30 YEARS OF CLIMATE CONTROL AND FACE CREAM

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The virus genus *Coccolithovirus* (*Cocco*: derived from Greek *kokkis*, meaning “berry” or “grain” referring to their shape and *Lith*: from Greek *Lithos*, meaning “stone”) is a group of large, double stranded DNA viruses that infect the globally important marine coccolithophorid *Emiliana huxleyi*. The first observation of virus-like particles in *E. huxleyi* was reported back in 1974 though they are now known to be one of the causative agents of *E. huxleyi* bloom demise. We have developed diagnostic molecular tools to analyse the dynamics of coccolithoviruses and their hosts during natural blooms. Virus infection of *E. huxleyi* increases production of the biogenic gas dimethyl sulphide (DMS), which has implications for climate feedback mechanisms. We have recently sequenced the 407,339 bp genome of one coccolithovirus and revealed that only 14% of the predicted genes confer any significant database homology. The genome encodes a range of unexpected genes never previously observed in a virus. Most notably are those involved in biosynthesis of ceramide, a sphingolipid better known for its role in face cream. Microarray analysis of potential genes on the virus genome will greatly enhance our understanding of the propagation of this unusual virus and why algal viruses have such large genomes.

## POSTER

### VIRAL DIVERSITY AND PRODUCTION DURING *TRICHODESMIUM* BLOOMS IN THE GULF OF MEXICO

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*Trichodesmium* spp. are significant primary producers and nitrogen fixing organisms in tropical oligotrophic marine environments. The dynamics of nitrogen fixation and the impacts of zooplankton grazers and viruses on nitrogen fixation rates of *Trichodesmium* were investigated in the eastern Gulf of Mexico. Changes in viral diversity and production were assessed during *Trichodesmium* blooms in the summers of 2002 and 2003. Using pulsed-field gel electrophoresis (PFGE), differences in viral diversity were detected between blooms, but little change in PFGE banding patterns was observed over the course of individual blooms. Viral production rates were estimated using a tangential flow diafiltration approach and were similar to viral production estimates for other oligotrophic marine environments. In incubation experiments during 2003, additions of *Trichodesmium* colonies did not significantly change the viral production rate compared to unaltered controls (Student' t-test,  $P > 0.05$ ). Viral production rates decreased throughout the course of the 2003 bloom, and the rate measured at the end of the bloom was significantly less than estimates from early in the bloom (one-way ANOVA,  $P < 0.05$ ). It appears that the diversity of viral communities and viral production rates associated with *Trichodesmium* blooms varies little over short time scales. However, over longer time scales, between blooms, viral diversity and viral production can vary greatly. This suggests that viral impacts on *Trichodesmium* fluctuate between distinct blooms of this important phytoplankter.

## ORAL

### HOW WELL DO CULTIVATED ALGAL VIRUSES REFLECT VIRIOPLANKTON GENOTYPIC DIVERSITY?

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The past few years has seen a dramatic increase in the availability of whole viral genome sequence data. Typically, viral genomes contain a disproportionately high number of unknown and hypothetical genes. Recent exploration of viral genetic diversity within metagenome libraries demonstrates that 60 to 70% of viral genes are unknown. Thus, viral communities contain the largest reservoir of unknown genes in the biosphere. To determine the extent to which fully-sequenced algal viral genomes represent gene diversity within Chesapeake Bay viroplankton, tBLASTX comparisons between whole algal viral genomes and a Chesapeake Bay viroplankton metagenome library were performed. The metagenome contained 363 BLAST hits to the ~48 kb cyanophage P60 genome at an expectation score of 10e-6 or lower. Fifty four percent of the P60 genome was homologous to sequences in the metagenome. Partial genome sequence from the ~45 kb photosynthetic region of cyanophage P-SSM2 showed similar homology to the metagenome. In contrast, only 4% of the ~330 kb Paramecium bursaria Chlorella virus was homologous to the Chesapeake Bay metagenome. Interestingly, BLAST hits were not randomly spread throughout the genomes, but were localized to specific regions. Homologs to the photosystem II core reaction center in P-SSM2 were especially well represented indicating this gene is widespread among cyanophages. The localized nature of BLAST homologies suggests that metagenome analysis can provide insight on the essential and broadly distributed genes among specific viral groups. The implications of using known algal viral genomes to understand the broader genotypic diversity of viroplankton will be discussed.

## ORAL

### ACCUMULATION OF GLUCOSAMINOGLYCAN ON VIRUS-INFECTED *CHLORELLA* CELLS

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Hyaluronan (HA) synthesis in chlorovirus PBCV-1-infected *Chlorella* cells was previously reported (DeAngelis et al., Science 278:1800-1803,1997). Chlorovirus CVK2 encodes a chitin synthase gene, *chs*, instead of hyaluronan synthase gene, *has*, and produces hairy chitin (CH) polysaccharide on the infected cell surface. Interestingly, a few isolates of chloroviruses contained both the *has* and *chs* genes and formed both HA and CH simultaneously on the infected cells (Kawasaki et al., Virology 302:123-131, 2002). CH is a polymer of  $\beta$ -1,4-linked GlcNAc that occurs in the exoskeleton of arthropods, in the cell wall of fungi and in various components of diverse invertebrates. Meanwhile, HA is a polymer composed of alternating  $\beta$ -1,4-GlcA and  $\beta$ -1,3-GlcNAc. HA is found in every tissue of animal body, especially rich in the vitreous body, the synovial fluid of articular joints and the intercellular space of the epidermis. Some pathogenic bacteria also produce HA as an extracellular capsule, a stealth material hiding from the host immune system. We will discuss about the biological significance of such glucosaminoglycan synthesis in the chroloviral infection and about the inter-relationship between *has* and *chs* on the chroloviral genomes.

## POSTER

DETECTION OF PBCV DNA AND VIRAL PARTICLES IN THE SYMBIOTIC SYSTEM *PARAMECIUM BURSARIA* – *CHLORELLA* SP.

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PBCV is a virus, which specific hosts are green algae – zoochlorella, living endosymbiotically in *Paramecium bursaria* (Ciliata, Protozoa) cells. Previous investigation showed that PBCV lyses zoochlorella isolated from *P. bursaria*, but zoochlorella inside the host cell never contain viral particles. The studies of these viruses have been mainly carried out on isolated and maintained in culture zoochlorella. This virus-containing system is an advantageous model to study life cycle and mechanisms of transmission and maintenance of the virus. We applied pulsed-field gel electrophoresis (PFGE) to study the system as a whole. PFGE profiles of *P. bursaria* total DNA have been characterized earlier (Rautian, Potekhin, 2002). The special feature of some of those profiles was a sharp band about 300 kb, which is exactly the size of PBCV genome. Applying Southern hybridization with the virus-specific probe, we have demonstrated that this band was, in fact, formed by the viral DNA. This means that large amount of nonintegrated viral DNA was contained in tight association with *Paramecium* cells.

## ORAL

ISOLATION AND CHARACTERIZATION OF A NOVEL PHAGE INFECTING A TOXIC CYANOBACTERIUM *MICROCYSTIS AERUGINOSA*

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*Microcystis aeruginosa* is a unicellular colony-forming cyanobacterium that produces cyclic heptapeptide toxins (microcystins) and forms dense blooms in eutrophic lakes and reservoirs all over the world. Due to the hepatotoxicity of microcystins, mass blooms of *M. aeruginosa* lead to serious threat for human health concerns. We succeeded in isolating a novel phage (Cyanophage Ma-LMM01) specifically infecting a toxic strain of *M. aeruginosa* (NIES-298) out of the Lake Mikata, Fukui Prefecture in Japan. The virion is not enveloped and has an isometric head and a tail complex consisting of a central tube and a contractile sheath. The head of Ma-LMM01 is ~86 nm in diameter; the tail complex consists of a central tube (~9 nm in width) and a contractile sheath (~24 nm in width, ~209 nm in length), provided with a multi-layered collar and a base plate. The sheath contracts to ~90 nm in length, then shows helical symmetry. Based on these morphological features and the host specificity, Ma-LMM01 is most likely belongs to the group Cyanomyovirus. Its latent period and burst size were estimated at 6-12 h and 50-120 infectious units cell<sup>-1</sup>, respectively. Although there were a few reports on isolation of phages infecting *M. aeruginosa*, Suttle (2000, in “The Ecology of Cyanobacteria”) pointed out the confusion in identification of their host cyanobacterial strains, i.e. incorrect classification. Hence, as far as we know, this is the report describing the first isolation of a phage infecting the toxic cyanobacterium *M. aeruginosa*. This study was financially supported by NEDO of Japan.